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Fourier Transform Infrared Study of Proteins with Parallel β-Chains

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Received April 7, 1987

Deconvolved and second derivative Fourier transform infrared spectra of the proteins flavodoxin and triosephosphate isomerase have been obtained in the 1600 to 1700 cm^{-1} (amide I) region. To our knowledge these results provide the first experimental infrared data on proteins with parallel β-chains. Characteristic absorption bands for the parallel β-segments are observed at 1626–1639 cm^{-1} (strong) and close to 1675 cm^{-1} (weak). Previous theoretical studies based on hypothetical models with large, regular β-sheets had suggested bands close to 1650 and 1666 cm^{-1}. Our new assignments were confirmed by band area measurements, which yield conformational information in good agreement with results from X-ray diffraction data. The spectra were compared with corresponding spectra of concanavalin A and carboxypeptidase A. The first contains only antiparallel β-segments, the second "mixed" β-segments, with some strands lying antiparallel and others parallel. None of the observed amide I band frequencies assigned to parallel β-chains occurs in the 1650 cm^{-1} region associated with helical segments.

A considerable amount of experimental and theoretical research has been done on the infrared spectra of proteins and polypeptides containing antiparallel β-strands (1-5). It is generally agreed that for this conformation the amide I mode, i.e., the backbone C=O stretching vibration, gives rise to one or more strong infrared bands in the 1625–1640 cm^{-1} spectral region and a much weaker band in the 1670–1680 cm^{-1} spectral region. Very little is known empirically about the corresponding characteristic infrared bands associated with the parallel β-conformation because (a) model peptides containing extended parallel chains would be difficult to prepare and (b) globular proteins with parallel β-strands by necessity contain a large amount of other conformations, such as helical sections, connecting the β-strands.

To the best of our knowledge, the infrared spectra of no polypeptides or proteins with parallel β-segments have been experimentally studied. Several theoretical calculations have been carried out to predict the frequencies that an infinitely long, all-parallel β-sheet should exhibit. The latest, by Bandekar and Krimm (6), predicts amide I components at about 1666 and 1650 cm^{-1}. The latter value is very close to the well-known characteristic frequency for helical segments (1-5). We have now observed experimental amide I component frequencies for the well-characterized globular proteins flavodoxin and triosephosphate isomerase. All β-strands of both proteins are aligned parallel to one another and are connected by either helical sections or by loops (7, 8). The observed frequencies for these β-segments are quite different from the predicted values. Analogous spectra of concanavalin A and carboxypeptidase A are presented for comparison. These proteins have antiparallel and mixed β-chains, respectively.

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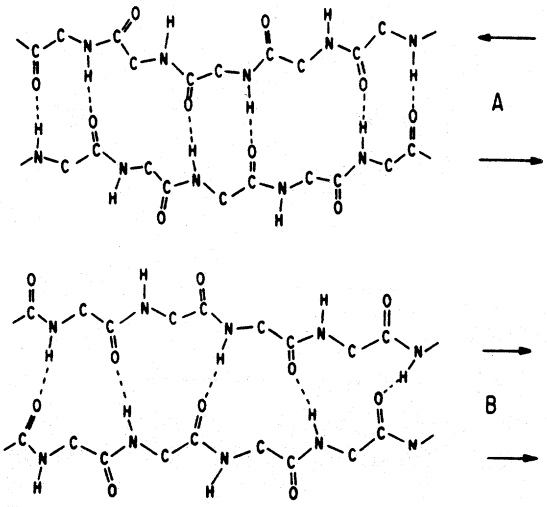

FIG. 1. Schematic representation of (A) antiparallel and (B) parallel β-strands. Side chains and hydrogen atoms attached to α-carbons have been omitted. The arrows point from N-terminus to C-terminus.

EXPERIMENTAL

A sample of flavodoxin was obtained through the courtesy of Professor Vincent Massey of the University of Michigan Medical School. Triosephosphate isomerase, baker's yeast (T-2507), was purchased from the Sigma Chemical Co.[2] The experimental procedure follows that previously reported for a number of globular proteins (9). The proteins were studied as 5% solutions in D_2O containing 0.1 M NaCl. D_2O was used as a solvent because H_2O absorbs very strongly around 1640 cm^{-1}, in the spectral region of interest. The spectra were obtained after complete D,H exchange had taken place in the peptide groups. The completeness of exchange was determined by the disappearance of the amide II band, as described by Timasheff *et al.* (1). The amide I frequencies observed in D_2O solution are slightly lower (5 cm^{-1} or less) than corresponding frequencies observed in H_2O (1, 5, 9, 10). All spectra were calculated from 4000 coadded interferograms collected on a Nicolet 7199 FTIR[3] spectrometer (9). The path length was 0.075 mm; nominal instrument resolution was 2 cm^{-1}. Second derivative spectra and deconvolved spectra were obtained as previously described (9). In particular, deconvolution was applied to a 450 cm^{-1} region of each spectrum (1800–1350 cm^{-1}). The values used for the parameters VF0 and VF1 (as defined by the Nicolet deconvolution algorithm) were 13.0 cm^{-1} for the full width at half height, and 2.4 for the resolution enhancement factor. Areas of deconvolved band components were estimated with the help of the Gauss-Newton iterative curve-fitting program ABACUS written in this Research Center by W. H. Damert (9). The deconvolved components were found empirically to have Gaussian envelopes. Excellent agreement was obtained between observed and calculated spectra after deconvolution (see below).

RESULTS AND DISCUSSION

Figure 1 schematically illustrates short sections of two antiparallel β-chains and of two parallel β-chains. Figure 2 shows a simplified rendition of the secondary structures of the four proteins: flavodoxin and triosephosphate isomerase contain β-structures with all chains parallel; the β-sheet of carboxypeptidase A has strands with "mixed" orientations; and concanavalin A has wholly antiparallel β-sheets (8). Figure 3 presents the original FTIR spectra

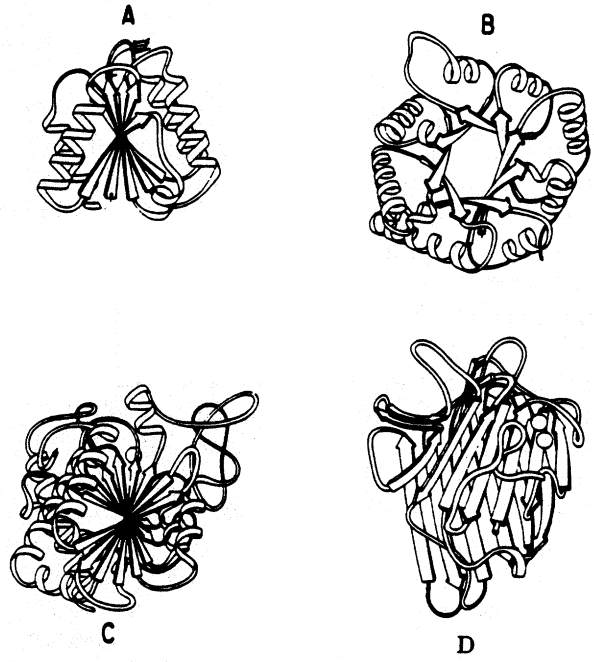

FIG. 2. Simplified representation of the peptide backbone structure of (A) flavodoxin (parallel β-strands), (B) triosephosphate isomerase (parallel β-strands), (C) carboxypeptidase A (mixed β-strands), and (D) concanavalin A (antiparallel β-strands). Reproduced, by permission of the publisher, from Ref. (8).

[2] References to brand or firm names does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

[3] Abbreviation used: FTIR, Fourier transform infrared.

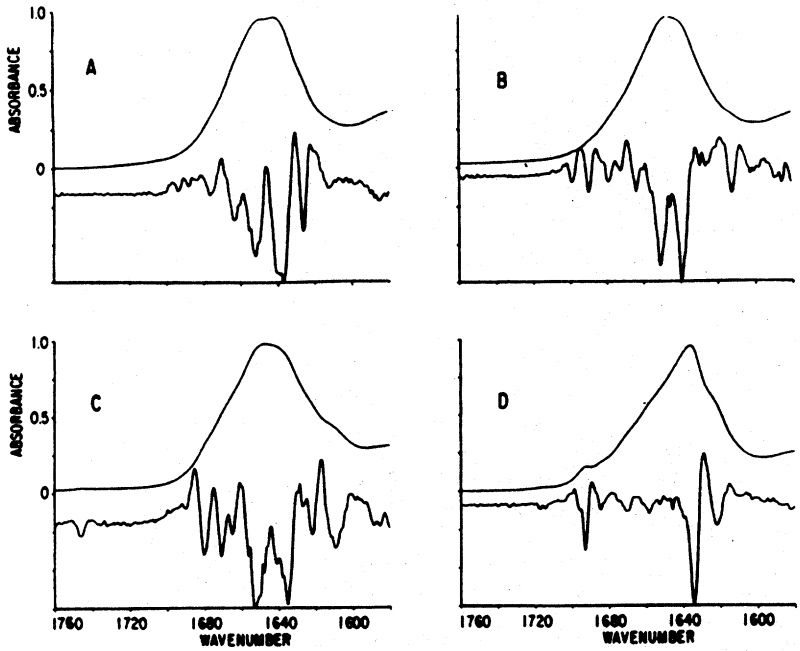

FIG. 3. Original and second-derivative spectra (all data unsmoothed) of (A) flavodoxin (parallel β-strands), (B) triosephosphate isomerase (parallel β-strands), (C) carboxypeptidase A (mixed β-strands), and (D) concanavalin A (antiparallel β-strands) in the amide I spectral region. Wavenumber (cm^{-1}) plotted vs absorbance in original spectra and vs absorbance/wavenumber^2 in second-derivative spectra.

and unsmoothed second derivative spectra of these proteins. Figure 4 gives the corresponding deconvolved spectra, including the component bands determined by curve fitting. Table I summarizes the observed frequencies for the α-helices and the β-strands.

The second derivative spectra in Figs. 3A and 3B reveal that the proteins with parallel β-chains, flavodoxin and triosphosphate isomerase, have strong bands in the 1620–1639 cm^{-1} range and a weak band in the 1670–1680 cm^{-1} region. These bands can be assigned to parallel β-strands by comparison of the relative band areas with structural data from X-ray diffraction studies (7), as described before (5, 9) and summarized in Table II. In addition, both proteins exhibit a strong band close to 1652 cm^{-1}, assigned to the several helical segments of these proteins. The second derivative spectra displayed in Figs. 3C and 3D (carboxypeptidase A and concanavalin A) demonstrate that proteins with mixed chains and with antiparallel chains have bands only in the same spectral regions as the proteins with all parallel chains. Carboxypeptidase A also exhibits a strong helix band close to 1652 cm^{-1}, as expected on the basis of X-ray data (7).

The deconvolved spectra shown in Fig. 4 confirm the information gained from the second derivative spectra presented in Fig. 3. All four proteins have components associated with β-strands in the 1620–1639 cm^{-1} and in the 1670–1680 cm^{-1} ranges. Again, all except concanavalin A have a strong component close to 1652 cm^{-1}, arising from helical segments. The remaining band components are associated with turns and with undefined sections (1, 5, 9).

Table I summarizes the observed frequencies and assignments of the components associated with β-strands and with helical segments. Table II gives the areas of the observed components as a percentage of the total amide I area, as compared to the percentage of helix or β-structure estimated from X-ray data by Levitt and Greer (7). The general agreement between

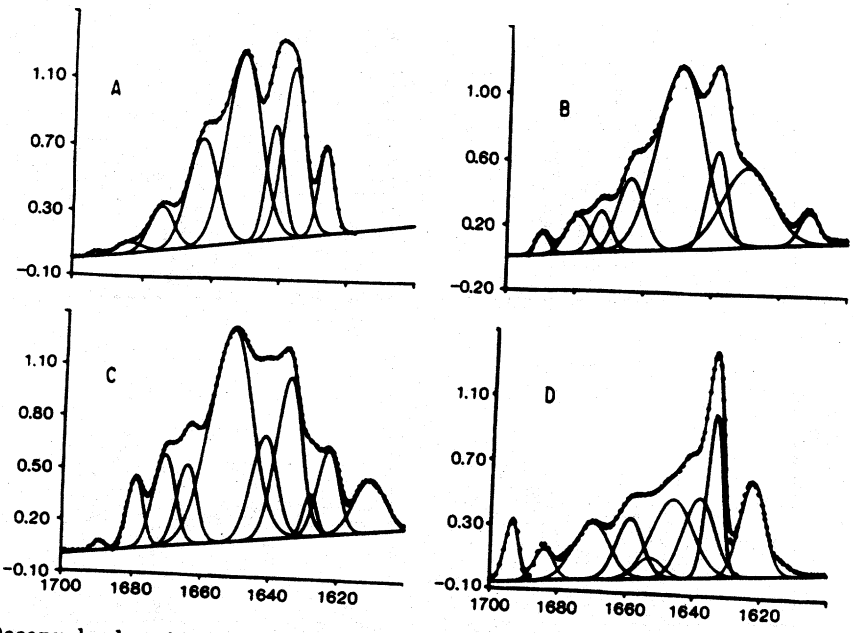

FIG. 4. Deconvolved amide I bands of (A) flavodoxin (parallel β-strands), (B) triosephosphate isomerase (parallel β-strands), (C) carboxypeptidase A (mixed β-strands), and (D) concanavalin A (antiparallel β-strands). Deconvolution parameters: VF0 = 13.0 cm^{-1}, VF1 = 2.4 (see text). Wavenumber (cm^{-1}) plotted vs absorbance.

band areas and X-ray results is quite satisfactory. In particular, the very good agreement concerning helical segments strongly suggests that the band near 1652 cm^{-1} is caused solely by the helical conformation; i.e., in contrast to theoretical predictions (6), there is no parallel β-component close to that frequency. The somewhat high value found for the relative area of the β-component of triosephosphate isomerase in comparison to the X-ray estimate is typical of proteins with substantial helix content (5). Apparently, in such proteins there are sections of extended chains which absorb at frequencies similar to the amide I frequencies of true β-segments (9).

A few words might be in place concerning the splitting of the low frequency components of the β-segments which are observed in the 1620–1639 cm^{-1} spectral region. The β-strands of globular proteins are usually quite short, 3 to 12 residues each (7, 8). The spectra therefore reflect short sequences of coupled oscillators which are expected to exhibit a series of bands instead of just the two bands theoretically expected for a polymer with indefinitely long β-segments (2–4, 9). The discrepancy

TABLE I

AMIDE I COMPONENTS ASSIGNED TO β-SEGMENTS AND HELICAL SEGMENTS

Protein	Orientation of β-strands	β	Helix	β
Flavodoxin	Parallel	1675	1651	1636, 1626
Triosephosphate isomerase	Parallel	1673	1652	1639, 1631
Carboxypeptidase A[a]	Mixed	1679	1652	1635, 1628
Concanavalin A[a]	Antiparallel	1671	1653	1639, 1634, 1623

[a] Data and assignments from Ref. (5).

TABLE II

COMPARISON OF RELATIVE FTIR BAND AREAS WITH SECONDARY STRUCTURE DATA BASED ON X-RAY DIFFRACTION[a,b]

Protein	% area of β-bands	% β-structure by X ray	% area of helix band	% helix by X ray
Flavodoxin	34	34	36	41
Triosephosphate isomerase	36	25	46	52
Carboxypeptidase A[c]	33	30	40	39
Concanavalin A[c]	60	60	4	2

[a] Measurement of FTIR band areas as a percentage of the total area of the amide I band is as described in Refs. (5, 9).

[b] X-ray data from Ref. (7).

[c] Data from Ref. (5), for comparison.

between our empirically observed frequencies for parallel β-chains (ca. 1635 and 1675 cm^{-1}), and those estimated by theoretical calculations (ca. 1650 and 1666 cm^{-1}) (6) is probably rooted in similar considerations. Recent preliminary calculations (H. Susi and D. M. Byler, unpublished work) indicate that a dipole-dipole type interaction force constant of ca. 0.3 mdyn/A between adjacent C=O groups of a single polypeptide chain will produce splittings of about 40 cm^{-1} between the in-phase and out-of-phase stretching vibrations, in rough agreement with our observed values for the high- and low-frequency components of bands associated with β-strands in actual proteins.

To summarize, we conclude that contrary to results suggested by theoretical calculations (6), in the case of globular proteins it does not appear to be possible to distinguish between parallel and antiparallel β-chains on the basis of amide I infrared frequencies. This observation, however, makes it possible to estimate the total β-structure content as well as the total helix content by resolution-enhanced FTIR spectra, no matter what the relative orientation of neighboring β-chains.

ACKNOWLEDGMENT

The authors thank Stephen McGady for assisting in obtaining the spectra of triosephosphate isomerase and flavodoxin.

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